The influence of mechanical resistance on the development and life period of cells.

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(Continued from page 157.)

3. *On differentiation in the fibrovascular bundle.*—It has already been pointed out (p. 154) that the effect of mechanical resistance to extension in the tissue adjoining the growing point is to delay the differentiation of that homogenous tissue into heterogeneous. It is also true that the same cause will delay the later changes which take place normally in the primary bundles by which some of their elements are developed into mechanical xylem and phloem.

Many young stems of various species of plants have received casts about internodes at such a time that the primary differentiation of tissue had proceeded so far as to mark off fascicular from fundamental, and such internodes in their subsequent growth have fallen far behind the neighboring free parts of the stem in progressive changes in the bundles; and it must be emphasized that this delay in development is not due merely to the fact that change could not take place because no cells were formed to undergo change. It will be seen that the cells already present before casting have within the casts retained their weak condition much longer than corresponding ones beyond the limits of the cast. Vicia faba has in all cases thickened none of the xylem parenchyma cells of the primary bundles within casts and formed no secondary tissue at the time when beyond the limits of the casts not only these primary elements had been thickened but a secondary zone of mechanical xylem ten to twelve cells wide had arisen. In Althaea tauriensis every bundle outside the omits of the casts develops many thick-walled cells before line appears in the bundles within the casts. Archangelica sativa and Myrrhis odorata thicken the elements in their primary bundles and form a wide band of thick-walled secondary xylem in normal parts of their stems before a thick-walled element appears in any bundle in the part of the stem confined by gypsum. A typical preparation of this plant serves also to show the effect of the confining cast on the thickening of the walls of vessels: an average bundle within the cast had

in a radial row twelve thick-walled vessels, twenty-eight thin-walled; above the cast in the same stem in an average bundle the thick-walled vessels were forty, the thin-walled twelve. A shoot of Sambucus nigra that had grown for several weeks with a cast around one of its internodes had when examined a secondary zone of cells within the cast as well as out of it. Within the cast and a little above the cast the number of cells in a radial row of this zone was eighteen, the same in each case. But in the former only three cells had thick walls, while in the latter there were six such. Similar results to the foregoing were obtained in Urtica dioica, Dahlia variabilis and Forsythia viridissima. No contrary effect was obtained in any plant.

In experimenting on the effect of pressure on the development of thin-walled phloem into hard bast the results will perhaps be more striking, since in the plants selected the hard bast is of primary origin and the casts were not applied till the very cells destined for the fibres were present in the thin-walled condition. Hence in such cases certainly the possible factor of the interference of the cast with cell-division and so with the final result is eliminated.

In the second or third epicotyledonary internode of Vicia faba the hard bast is formed when the whole plant has produced five or six internodes. If however the internode in question is put into a cast before secondary growth begins, no hard bast is formed there till the plant has reached twice the development, that is, till about the time it blossoms. Before this time the xylem within the cast will have thickened several or many walls and outside the cast mechanical xylem and phloem will have been present four or five weeks. Two plants of Althæa tauriensis, which had grown well for eighteen days after a lower internode in each had been encased in gypsum, showed within the confined internodes only thin-walled cells, while above and below the casts hard bast was present as well as thick-walled xylem. Other individuals of the same species, which after similar treatment grew for fifty days, had begun within the casts to thicken the walls of the bast cells. Urtica dioica, grown for twenty-three days after application of casts, had within the casts no thick-walled bast, but considerable in the normal adjoining internodes. Other individuals, after growing under the same conditions of treatment as these for fifty days, had developed hard bast

of considerable but less than normal thickness. Archangelica sativa, examined after twenty days' growth with a cast around a segment of its stem, had outside the cast strong mechanical bast, inside the cast only thin-walled cells. Dahlia variabilis when grown with a cast around the stem develops hard bast outside the cast several weeks before it appears within; the same is true of Ricinus communis, Forsythia viridissima and Pterocarya fraxinifolia.

In all these plants in which the delay in the formation of thick-walled xylem and phloem has been considered, there has been an actual extension of the period between the origin of the cells and the assumption of their permanent condition. This is proved by the fact that all of the species that have been grown for a very long time surrounded by casts, and these include all those named above except Althæa and Archangelica, have shown the xylem and phloem cells slowly increasing in thickness of wall after the corresponding elements in unconfined parts of the stems had reached, so far as could be determined, their mature condition.

4. *On the time of cork-formation.*—No experiments were made directly on the formation of cork. The results are thus merely incidental and furnish no information except on the time of the beginning of cork-formation, as influenced by the enveloping casts of gypsum. Whether the influence of the casts is due to the pressure which is soon developed by the effort of the confined tissues to expand, to the protective influence of the envelope, or to some other cause is left undetermined. It seems probable, however, as will appear in the general summary at the close of this paper, that it is the pressure exerted which is the influential factor.

In all of the plants that have given results under this heading, cork-formation appears more tardily within the limits of the casts than outside. This has proved true for Forsythia viridissima which forms phellogen from the epidermis; for Phytolacca dioica and Dahlia variabilis which form phellogen from the first hypodermal row of cells; and for Melianthus major in which the phellogen appears in the innermost part of the cortex.

Effect of mechanical resistance on the permanent condition assumed by cells.

It requires no series of experiments to convince one that if a part of an organ in which primary extension is not com-

plete be so encased that it cannot grow farther, the cells must retain a smaller size than normal. The matter cannot, however, be so easily disposed of; the question of the interaction of the tissues, each striving to expand, still remains. We will consider first the effect on the division of cells; second, the manifestation of turgor between tissues; third, the definitive size of cells; and fourth, the thickness of membrane.

The results of my experiments in those cases where the material was suitable to the purpose have coincided with those obtained by Pfeffer[9] in the roots of seedlings in which he found that cells behind the growing point and in the elongating zone divided after the zone was put into a cast. The cells would thus be found shorter (but more numerous) than they were when the cast was applied. Yet this division without expansion does not progress very far, and it is only the cells that are near the stage of division before being put into the cast that divide within the cast. In cross-sections no division subsequent to the application of the cast has been found. It should be stated, however, that my preparations were not made to give, and were not often of a nature to give exact relations in this direction.

The displacement of tissues due to resistance to growth has been recorded in Aristolochia sipho by De Bary.[10] In this plant the pith is compressed by the approach of the fibrovascular bundles toward the center, the impelling cause being thought to lie in the resistance of the leathery cortex. A similar movement of the fibrovascular bundles toward the center has been induced in many of my plants by the resistance of the cast. The conditions for this displacement are that the gypsum must be laid around the stem before the fibrovascular zone has formed a bridge of mechanical tissue, that the pith contains intercellular spaces or has lost the most of its turgor, that the cortex is in an active condition and does not possess large intercellular spaces. Thus the pith has been compressed in Vicia faba, Urtica dioica, Archangelica sativa, Myrrhis odorata, Curcubita pepo, Melianthus major and Ricinus communis. When such a displacement of tissues has taken place, the pith is more or less distorted or crushed; the elements of the bundles are radially elongated and the vessels generally collapsed; the cortical cells are radially elongated and often assume the shape of palisade cells.

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[9]Pfeffer: Druck und Arbeitsleistung, 127.
[10]De Bary: Vergleichende Anatomie, 549.

The explanation for these changes is not difficult to find. The pith is the nearest of any tissue to the close of its growth and has the smallest turgor. The cortex is at the stage of its most rapid extension, and every cell dilates in the direction of least resistance. The young bundles grow also, but the amount of their extension is much less at this time than that of the cortex, consequently they do not check the inward pushing of the latter but are carried by it in against the pith whose cells are thus crowded closely together. This movement continues till the intercellular spaces are filled, or till the pre-existing pith-cavity is closed up, when an equilibrium is established and the cortex grows no farther. Displacement does not always end here, however, for the force of growth in the primary or secondary meristem of the vascular ring may prove sufficient to crowd back the cortical cells, often causing them to show wavy walls and in some plants, as Ricinus communis and Eryngium planum, actually crushing them.

The foregoing paragraph furnishes the key to the understanding of the definitive size attained by cells of various tissues when the resistance to growth is made effective before primary extension has ended. Thus although the cells retain longer than in normal growth their capability for extension, they must of necessity reach a time when that capability is lost. With a stem within a cast the pith-cells generally are found to use the least of any tissue the room for extension furnished by the intercellular spaces. The size which they attain relative to their normal size is very nearly the same which they had when the gypsum was laid around the stem. Thus in Archangelica sativa, which had been encased when young, the pith-cells were found to have one-half the diameter of those above and below the cast, though none of these cells had divided meanwhile. A similar result with a varying proportion in the size was obtained in all the plants experimented with, ten species in all.

Precisely similar results were found in the cells of the cortex, except that they nearly always expand more within the cast than do the pith-cells. In the cortex, however, the power of growth is retained both normally and within casts so much longer than in the pith that there were but few of the plants under experiment that showed cortical cells in their definitive condition. It is true, nevertheless, that the

definitive size of these cells would be the same as that possessed several weeks after the application of a cast, for in that period the cortex has reached the full extension allowed by the circumstances and any subsequent expansion will be confined to the bundles. In this case, then, we may reckon the size of the cortical cells after the cast has been around the stem for several weeks as the definitive size; whence it follows, that, like the pith, the cortical cells will reach their definitive state with longitudinal and cross-diameter much less than in normal growth.

De Vries and Krabbe in the writings already cited record the fact that the elements of the xylem, when growing under strong external pressure, will not attain their normal size. My experiments have confirmed this result in every plant used. The difference between the size attained and the normal size differs in various species. In Eryngium planum it was found in one plant to be as two to three; in one plant of Pterocarya fraxinifolia as one to two.

Not only the xylem part of the bundle produces elements of smaller size but the phloem also; though a smaller size for the cambium cells could not be demonstrated. These Krabbe found in his experiments to retain their normal size under all pressures; and it will be remembered as already cited in these pages that Pfeffer found the size of the meristematic cells of the growing points of roots and stems to be unchanged when growing in gypsum casts.

Regarding the ultimate thickness of membrane attained by cells growing against pressure, it may be said that the thickness of wall in undifferentiated fundamental tissue does not seem to be decidedly affected. There are many cases known where the cortical cells, for instance, some time after elongation has ended, thicken up their membranes considerably. Such membranes have in my experiments never been found to become thicker within the casts than normal, nor do they seem to remain much thinner. It is to be understood here that in this group of experiments the casts were applied when the stems were very young and while fundamental tissue was in its primary thin-walled condition; the subsequent differentiation into mechanical cells is not referred to, but only the even thickening of the parenchyma as it assumes its definitive condition. This thickening seems to progress within the cast as well as out of it though the cells within

may come far short of attaining their normal dimensions. As soon, however, as the development of mechanical cells be considered, we shall find, as will soon be pointed out, a profound effect produced by the cast. It must be indicated in this place that the question that has just been discussed—the influence of pressure on the thickening of the walls of fundamental parenchyma—may not be answered for every plant by my experiments. In all of my plants the normal thickness attained by this tissue is slight and hence a small variation caused by the cast would be difficult to distinguish. It is possible that in plants in which the fundamental parenchyma forms thicker walls than those used in my work, the effect of casts would be apparent in preventing the normal increase in thickness. It is not at all probable, however, that the opposite will be found true, i. e., that cells will form thicker walls than normal when pressure prevents them from attaining their full size. Precisely this assumption was, however, made by Wortmann.[11] He wound the stems of seedlings with twine and found upon examining them after they had been in these bandages for several days, that the cortical cells had thicker walls than normal. This result, it need not be said, seems contradictory to all of mine obtained by the use of casts. It can, however, I believe, be reconciled.

In the first place, it makes a great difference whether the growth be confined by a twine bandage or by a gypsum cast. In the former case the peripheral cells grow out between the strands, producing considerable distortion always accompanied by a good increase in the thickness of neighboring cell-walls. This has been demonstrated by my experiments on Ricinus communis and Phaseolus multiflorus, the latter being one of the plants used by Wortmann. When these plants, however, were encased in gypsum, no such distortion occurred, nor were any cell-walls, as Wortmann found them, greatly thickened, the same result that a dozen other species of plants have given.

In the second place, the first evident effect of confining the extension of young tissue is the closing up of intercellular spaces. The result is a collenchymatous tissue; for at the angles of the cells the two layers of the membrane are appressed and although the thickness has actually nowhere increased, one must look carefully to escape deception.

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[11] Wortmann, Beiträge zur Physiologie des Wachsthums. Bot. Zeit. **47**: 286. 1889.

In the third place when tension is induced within tissues a thickening of the cell-membrane follows.[12] This is shown slightly in some plants in which the cortical cells elongate toward the center of the stem after the application of a cast. It is still more apparent when within a cast a few cells of the cortex die and the neighboring cells crowd in to fill the space. It is shown also in all my preparations at the limits of the casts where great tension has arisen between the confined segment of stem and that portion just outside the cast that is striving to expand.[13] All these cases, however, lie outside the question as discussed by Wortmann.

If now we return to the particular case of Phaseolus multiflorus, each of several individuals has shown that the peripheral cell-walls are much thickened where the coils of the twine bandage have caused distortion. In this plant also there are large intercellular spaces in the cortex at a very early age even before the elongation of the hypocotyl is completed. The bandage of twine or gypsum causes these intercellular spaces to be closed within a few days and as a result there is at the angles of the cells a double thickness of wall, merely an *apparent* thickening. There was only one case in which there seemed to be a possibility that the cortical cells had abnormally increased in thickness of membrane within the cast. Here these cells had elongated considerably toward the center of the stem, and if the membranes were slightly stronger the change would probably be accounted for by the tension called forth. But in this plant it is not certain that the membranes had so thickened; since they were no thicker than sometimes found in very young normal individuals. It can at least be said that Phaseolus multiflorus furnishes no illustration of Wortmann's theory that membranes increase in thickness more than normally when their full extension is prevented by mechanical means. All of the plants used in my experiments have contributed to an opposite conclusion. Moreover, Pfeffer, in his experiments with roots and stems of seedlings, noticed no unusual thickening within the segments enclosed in gypsum.

The outer pith-cells in many of the species recorded in this article become normally thick-walled to such an extent that

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[12] Hegler's work as reported by Pfeffer, Berichte d. k. sächs. Gesellschaft der Wissensch., demonstrates the fact that tension increases mechanical tissue.

[13] For details in these experiments see Newcombe: The effect of mechanical resistance on the growth of plant tissues. Leipzig, 1893.

they pass into mechanical tissue. In no plant have these cells shown thicker walls within than without the casts, and where it can be determined that they have reached their definitive condition they are thinner walled within the casts than normally. Vicia faba and Melianthus major have illustrated the truth of the last statement. In these two plants, the stem, after being confined for several weeks within gypsum, was released and grew for weeks afterward. When sections were made the outer pith-cells were found thinner-walled than normal, though they had reached their definite condition. That they had reached their definite condition was determined from the examination of several individuals at different ages, and thus the time when development ceased could be inferred.

That the xylem elements remain definitely thinner-walled when growing under pressure was determined by Krabbe *(l. c.)* for trees. None of my plants have indicated a contrary result and the two species in which it could be certain that the definitive condition had been reached corroborated Krabbe's view. Melianthus major and Vicia faba have each in several instances been released from casts and allowed to grow subsequently. The xylem elements that had been formed while the casts were present could be easily distinguished by their thinner walls from those formed subsequent to the removal of the casts.

(To be concluded.)

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